Supplemental Document

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Organelle-specific phase contrast microscopy (OS-PCM) enables facile correlation study of organelles and proteins: supplement

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OS-PCM ENABLES EASIER AND SIMPLER CORRELATION STUDY OF ORGANELLES AND PROTEINS: SUPPLEMENTAL DOCUMENT

Cell handling, transfection and drug treatment

Cos-7 cells were purchased from the Cell Bank of the Typical Culture Preservation Committee, Chinese Academy of Sciences. Cos-7 cells were cultured in DMEM medium (GIBCO) supplemented with 10% fetal bovine serum (GIBCO) and 1% penicillin-streptomycin. The cells were maintained in a cell culture incubator at 37°C with 5% CO2 concentration. To label DRP1, transfection reagent mCh-DRP1 was used. mCh-DRP1 was obtained as a gift from Gia Voeltz (Addgene plasmid #49152). For the transfection experiment, cells were seeded in 50 mm glass-bottom culture dishes (WPI FluoroDish) and cultured to 60-80% confluency 24 hours prior to transfection. Lipofectamine 3000 was used for transfection. Lipofectamine 3000 (Invitrogen) was diluted in 150 µl Opti-MEM(Gibco) at a ratio of 3.75 µl Lipofectamine 3000 per 150 µl Opti-MEM. 2.5 µg mCh-DRP1 (Addgene) and 5 µl P3000TM (Invitrogen) were diluted in 150 µl Opti-MEM. The diluted mCh-DRP1 was then added to the diluted Lipofectamine 3000, mixed gently, and incubated for 10-20 minutes. The mixture was added to the culture dish and incubated for approximately 8 hours before imaging, maintaining the cells in a cell culture incubator at 37°C with 5% CO2.

For the DRP1 induction experiment shown in Figure 5 and 6, 4uM Ionomycin (SQ23377, MedChemExpress) was added to the culture dish and placed in a 37°C incubator for 30 minutes before imaging.

Supplementary figures：

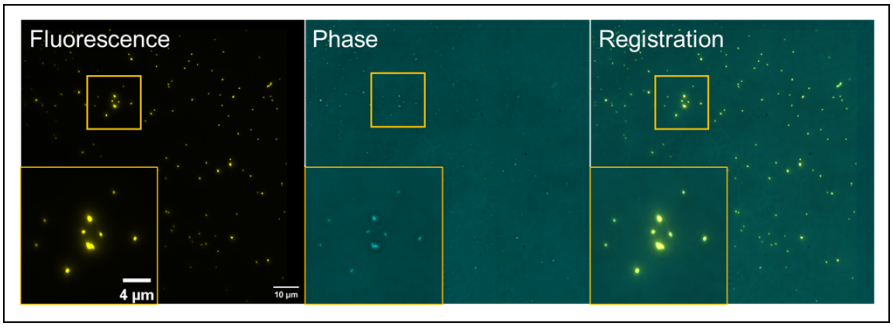


Fig. S1 Image registration using fluorescent beads.

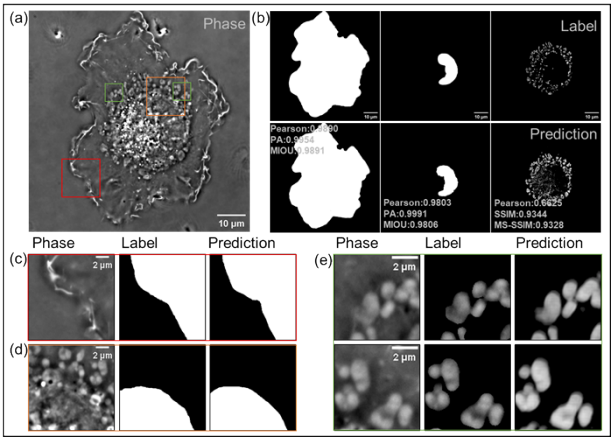
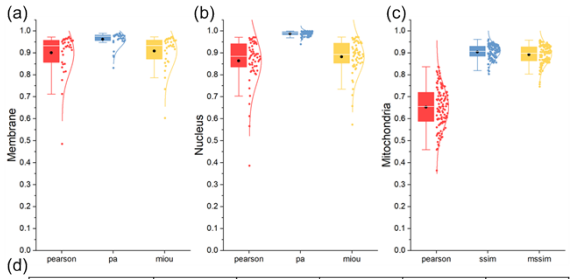


Fig. S2 Recognition of three cellular structures by CNN networks. (a) Phase image. (b) Label and prediction and of membrane, nucleus and mitochondria. (c). Zoom-in view (orange box) of image.



(d)

	Pearson	PA	MIOU	SSIM	MSSIM
Membrane	0.90	0.96	0.91		
Nucleus	0.86	0.99	0.88		
Mitochondria	0.65			0.90	0.89

Fig. S3 Quantitative evaluations of the prediction for membrane, nucleus and mitochondria. For cell membranes: Pearson =0.90 PA=0.96 MIOU=0.91 Details about those metric can be found in (d).

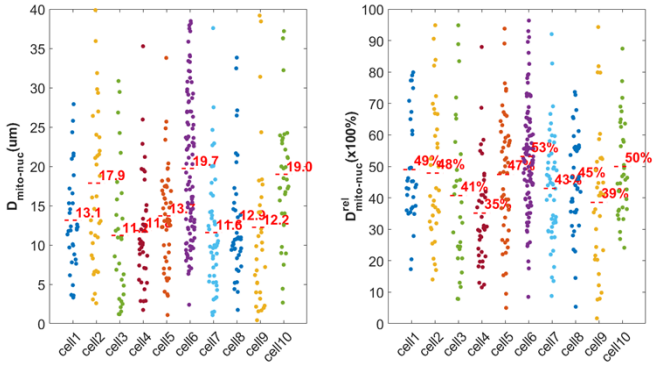


Fig. S4. Distributions of the absolute (left) and relative (right) distances between mitochondria and the cell nucleus for 10 cells studied in this work.

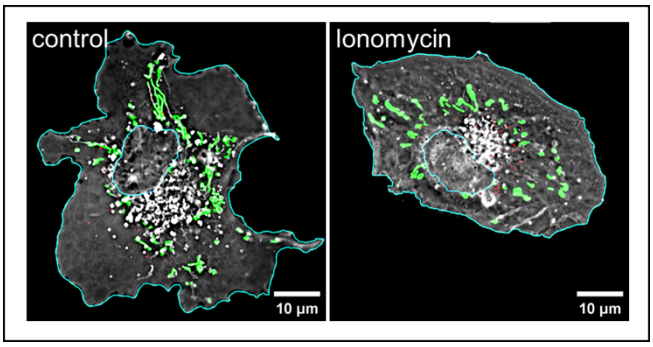


Fig. S5 Another example of cell images showing DRP1 and mitochondrial changes during apoptosis (same color representations as Fig. 2 in the main text).